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Research Article

Diagnosing Sweet Potato Aerial Vines of Two Semi Commercial Varieties for Virus's Accumulation from Production Sites in Western Highlands Province; Papua New Guinea Prior to Virus Elimination

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Abstract

A wide range of diseases and pathogens have been recorded on or associated with sweet potato in Papua New Guinea (PNG) however; there is little documented information on the viruses present in commercial production sites in Western Highlands Province (WHP) of PNG, not to mention their impact on production. Most diagnoses have been made on visual field symptoms without resort to standard virus detection methods, partly because of poor access to new technologies. The initial process to producing pathogen tested (PT) planting materials are diagnosing them and knowing what viruses present and biosecurity threats are existing in the region.

Two sweet potato varieties commonly grown for commercial purpose; Korowest and Rachael were selected for this study. Detection of *Sweet potato feathery mottle virus* (SPFMV) (34%) and *Sweetpotato caulimo-like virus* (SPCaLV) (9%) were detected either in single or dual infections using the herbaceous indicator plant; *Ipomea setosa* for virus transmission and assaying symptomatic leaves using the CIP NCM-ELISA Kit. A prominent feature of the results was the high incidence of virus's accumulation in the lower vegetative part of the plant (table 1) compared to low incidence accumulation in the top vine shoots. This indicates the use of aerial shoot tips as the best option for virus elimination methods as well as a better option for growers to use if the planting materials are not pathogen tested (PT). Apparently, commercial growers in that region are already using non PT vine shoots to cultivate sweetpotato and this study (table 1) confirms that shoots have lower incidences of viral accumulation compared to lower vegetative vines.

Keywords: Sweet Potato; Aerial Vines; Highlands Province; Virus Elimination

Introduction

Viruses are widely considered to be of great economic importance in sweetpotato production [1.2]. A survey of scientists from less developed countries rated viruses as the top priority (Fuglie 2007). Notably, however, no farmers in the 2014 PNG highlands survey mentioned viruses though a large proportion of old gardens showed foliar symptoms consistent with viral infection. This reflects the fact that symptoms of viral infection can be subtle and develop over a prolonged period with little or no direct symptoms on the storage roots other than yield decline which is likely to be attributed to pests because of their greater appearance. Related to this, the concept of a plant pathogenic virus, that has no signs, is relatively unfamiliar to many farmers so it not being mentioned is likely to reflect this fact. The availability of molecular detection methods has led to rapid advances in sweetpotato virus knowledge and at least 30 viruses of sweetpotato are known [1] some with multiple strains (Dolores, Yebron, and Laurena 2012). Yields of virus-infected sweetpotato plants are often severely affected, reduced by as much as 80-90% [1]. Though insects such as aphids such as *Aphis gossypii* and whiteflies including *Bemisia tabaci* can transmit viruses [1], propagation material is the chief means of viral spread [2]. Foliar symptoms of virus infection include leaf distortion, strapping and crinkling, mosaics, vein clearing, brown blotches and general stunting and chlorosis (Mbanzibwa., *et al*. 2014). These symptoms were significantly more frequently seen in old rather than new gardens reflecting the time available for plant-

to-plant transmission and buildup of infection levels [3]. Further diagnosis would confirm this.

Viruses are obligate pathogens that cannot be cultured outside of the host. They are too small to be detected by standard light microscopy and, for a long time, detection and identification relied on the use of electron microscopy and indicator plants, such as *Ipomoea setosa* in the case of sweetpotatoes. However, there was a need to develop sensitive virus diagnostic methods, especially to enable the production and dissemination of virus-free planting material for the international exchange and also for distribution within countries. The International Potato Center in Peru has been leading this research and has developed serological tests using ELISA as well as protocol for the detection of viruses using, PCR, the polymerase chain reaction (Salazar and Fuentes 2000).

Virus detection is a routine work for virus-free planting material production and safe movement of germplasm. Serology or other molecular diagnoses are expensive for many developing countries. *Ipomoea setosa* is a nearly universal sensitive indicator plant for sweetpotato viruses, which is used for graft-transmitted virus detection. Current international guidelines document that graft indexing successfully reveals most sweetpotato viruses [4]. Therefore, research institutes and seed enterprises of developing countries could benefit from using this technique for routine monitoring of planting materials in an inexpensive way without employing highly skilled manpower.

In this study, it was aimed to use standard diagnosing procedures using the indicator plant Ipomea setosa for sap transmission of virus from uncleaned sweetpotato specifically selected for semi commercial production to record virus symptoms and then to detect the type of virus and its accumulation using NCM-ELISA prior to virus cleaning process.

Materials and Method

Sweetpotato vine cuttings

Rachael and Korowest are two semi commercial sweetpotato cultivars locally grown within production sites in the Western Highlands Province of PNG and shipped out to markets around the country. Selection of the sweetpotato cuttings were done randomly based on samples exhibiting diseased symptoms during site visitation by Fresh Produce Development Agency (FPDA) staff in 2016. Samples were delivered to the National Agricultural Research Institution (NARI) in Aiyura, Eastern Highlands Province for producing and making available clean planting materials through the pathogen testing process. Prior to cleaning the pathogens, the samples were diagnosed for viruses.

The potting mix ratio used for sample establishment was locally available top soil, river sand and chicken manure (3:2:1). The *Ipomea setosa* seeds were imported from Australia and cool stored for virus indexing procedures.

Virus indexing

Sweetpotato aerial vines of Rachael and Korowest; shoot tip, mid vine and base vine were grafted onto the indicator plants by either terminal graft or side grafting method as described in the PT Manual (2013). Each sample was grafted onto at least three indicator plants. The method entailed cutting a 5 cm section containing at least one node from the vine sample and shaping the base of the section into a wedge (scion). The apex of the indicator plant was cut off to create a rootstock containing at least three leaves. The scion was then inserted into a 1.5 cm lateral slit in the rootstock stem. The graft junction was secured with the plumbers' tape. Healthy non-grafted indicator plants were maintained as negative controls. All plants were kept in an insect-proof screen net and watered weekly. Symptoms recording, and serological diagnosis were done for the third and sixth week after grafting from the grafted indicator plant using the CIP NCM-ELISA kit.

Nitrocellulose membrane enzyme-linked immunosorbent assay

Symptomatic leaf samples from graft-inoculated *I. Setosa* were assayed for *Sweet potato feathery mottle virus* (SPFMV), *Sweet potato mild mottle virus* (SPMMV), *Sweet potato chlorotic stunt virus* (SPCSV), *Sweet potato chlorotic fleck virus* (SPCFV), *Sweet potato virus G* (SPVG), *Sweet potato mild speckling virus* (SPMSV), *Sweet potato caulimo-like virus* (SPCaLV), *Sweet potato latent virus* (SPLV), *Cucumber mosaic virus* (CMV) and *Sweet potato C-6 virus* (C-6) using standard NCM-ELISA kits obtained from the International Potato Center (CIP), Lima, Peru. In addition to polyclonal antisera to the above 10 viruses and goat anti-rabbit conjugated antibody, the kit also contained membrane strips prespotted with sap from virus- positive (positive control) and healthy control plants (negative control). To test leaf samples with NCM- ELISA, the protocol was followed according to the manufacturers' instructions and visual assessment for varied degrees of purple colour development on the blot was used to identify virus-positive samples.

Results

Field assessment of virus disease

The majority of samples collected from sweet potato growing areas in WHP exhibited a diverse array of symptoms characteristic of virus infection. Symptoms observed in field samples from areas growing commercial cultivars (Rachael and Korowest) were not severe and predominately consisted of leaf distortion, leaf curl and chlorotic spot (Figure 1).

Virus indexing

Typical sweet potato virus-like symptoms were observed on grafted *I. Setosa*. Indicator plants grafted with samples showed vein clearing, stunting, mottling and chlorotic spots whilst negative controls showed nil symptoms of virus symptoms figure 2.

Figure 1: Sweet potato leaves exhibiting chlorotic spots.

Figure 2: Symptomatic leaves samples of *Ipomea setosa* grafts of Rachael and Korowest 3-6 weeks after grafting. Main symptoms recorded were a) vein clearing, b) leaf distortion (rugosity, curling), c) mottling, d) chlorotic spots and e) healthy *I.setosa* plant.

Nitrocellulose membrane enzyme-linked immunosorbent assay

A total of 2 viruses were detected in the 96 samples from symptomatic sweet potato plants collected from the seven commercial production areas visited in WHP. Respective vine samples from both varieties reacted positively to antisera for SPFMV (34%) and SPCaLV (9%) (Table1). From individual varietal vegetative part assessment; SPFMV was highly detected in mid vines of Rachael in 100% of samples and lower detection in shoot (50%) and base (33%) after third week of grafting (Table 1). SPFMV was detected as a dual infection at low incidence in Korowest shoot (25%), but had higher incidence detected with SPCaLV in mid vine (67%) and (50%) in base vine. The incidence and distribution of viral transmission in respective varietal vegetative parts had similar patterns of high detection in mid vines of the samples. All samples tested positive for the presence of one or two viruses (Figure 3).

	Number of samples and incidence (%) testing positive for a specific virus[a]												
	Variety	Vegetative part	Total number of samples	SPFMV	SPCaLV	SPCSV	SPVG	SPCMV	SPLV	SPMMV	SPCFV	SP C-6	SPMSV
3rd wk	Rachael	Shoot	6	3 (50)	0	0	0	0	0	0	0	0	0
3rd wk	Rachael	Mid	6	6 (100)	0	0	0	0	0	0	0	0	0
3rd wk	Rachael	Base	9	6 (67)	0	0	0	0	0	0	0	0	0
6th wk	Rachael	Shoot	6	0 (0)	0	0	0	0	0	0	0	0	0
6th wk	Rachael	Mid	6	6 (100)	0	0	0	0	0	0	0	0	0
6th wk	Rachael	Base	9	3 (33)	0	0	0	0	0	0	0	0	0
	Total		42	24 (57)	0	0	0	0	0	0	0	0	0
3rd wk	Korowest	Shoot	12	3 (25)	0	0	0	0	0	0	0	0	0
3rd wk	Korowest	Mid	9	0	6 (67)	0	0	0	0	0	0	0	0
3rd wk	Korowest	Base	6	0	3 (50)	0	0	0	0	0	0	0	0
6th wk	Korowest	Shoot	12	6 (50)	0	0	0	0	0	0	0	0	0
6th wk	Korowest	Mid	9	0	0	0	0	0	0	0	0	0	0
6th wk	Korowest	Base	6	0	0	0	0	0	0	0	0	0	0
	Total *Overall assessment 96*		54	9 (17)	9 (17)	0	0	0	0	0	0	0	0
			33 (34)	9 (9)	0	0	0	0	0	0	0	0	

Table 1: Serological detection of viruses in indicator plants grafted with sweet potato (*Ipomoea batatas*) samples collected from 7 commercial production sites in WHP, PNG.

a: Viruses detected by nitrocellulose membrane enzyme-linked immunosorbent assay

b: Values in parentheses are the incidence as a percentage

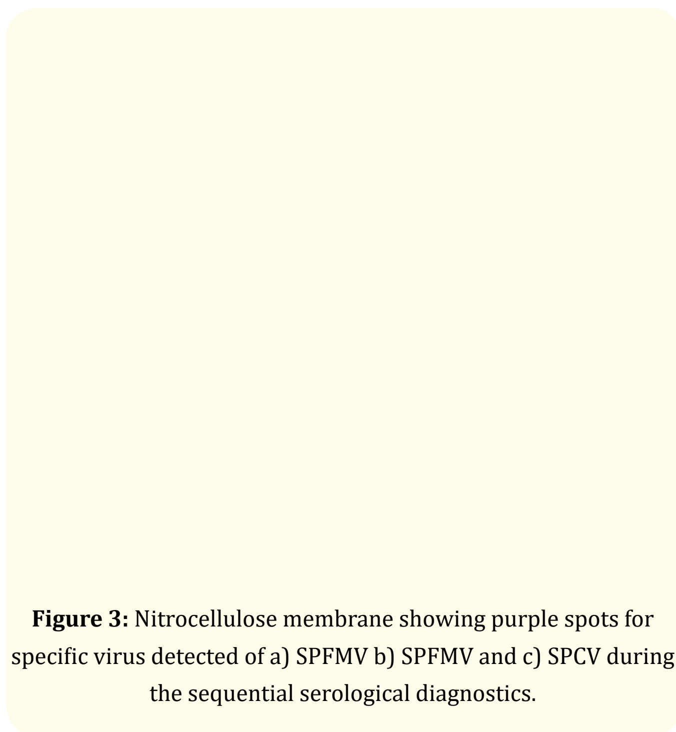

Figure 3: Nitrocellulose membrane showing purple spots for specific virus detected of a) SPFMV b) SPFMV and c) SPCV during the sequential serological diagnostics.

Discussions

Virus accumulation in many sweet potato cultivars is low and direct virus detection from sweet potato field samples is unreliable (Karyeija., *et al*. 2000). Therefore, grafting onto *I. Setosa*, nearly universal indicator plants for sweet potato viruses, is used to boost virus titre and leaves of grafted *I. setosa* are subsequently used in virus testing [4].

This study is the first of four comprehensive processes in the ongoing effort for clean seed scheme in supporting commercial sweetpotato production in PNG highlands. Detection of virus incidence of sweet potato in all major sweet potato growing areas in WHP provides an understanding on the viruses present and the likely risks associated with yield decline. Sequential diagnoses of plant samples indicated presence of *sweetpotato feathery mottle* (SPFMV) and *sweetpotato caulimo- like virus* (SPCaLV) in WHP (Figure 2a, b, c and d) and serological testing (Figure 2). A prominent feature of the results was the high incidence of viruses in the lower vegetative part of the plant that is used when growers run out of shoots (Table 1). A high level of viral incidence was speculated through continuous use of uncleaned planting materials spreading viruses by vectors; Aphids and whitefly from host plants given the diversity of sweetpotato varieties grown locally in the commercial sites. Apparently, commercial growers are already using shoots to cultivate commercially and from this study, it can be confirmed (Table 1) that shoots have lower incidences of viral transmission compared to lower vines. Farmers in these areas often grow sweet potato throughout the year and tend to favor the cultivation of their own local varieties over previously cleaned commercial cultivars. Cultivation of these local varieties may perpetuate *Sweetpotato feathery mottle virus* (SPFMV) is the most widespread and important virus of the c. 20 viruses detected in sweetpotato. It occurs worldwide wherever sweetpotato is grown. Different strains of the virus, based on the symptoms, have been recognized: russet crack (RC) causing characteristic symptoms on tuberous roots; common (C) strain and the severe (s) strains [5]. SPFMV can cause considerable yield reduction in sweetpotato and experiments have shown that virus-free sweetpotato plants yield 20 to over 100% more than infected plants (O'Sullivan., *et al*. 2005). SPFMV interacts with SPCSV to cause SPVD complex. So far, SPCSV has not been recorded in PNG [6-12].

Moreover, SPFMV is often present at a concentration below the limit of detection by ELISA [2] and, in those cases, can be detected only by grafting onto *I. setosa* instead of serological assay (Gutiérrez., *et al.* 2003). This is confirmed in table 1, figure 1a, b, c, and d) and serological testing in figure 2a, b, and c.

Conclusion

In this study, the accumulation of sweetpotato viruses in aerial vines was higher in the basal nodes compared to the shoot nodes indicating the use of top shoots as a better option when using non pathogen tested materials. This result also implies that viral transmission downwards affects yield during crop growth and development as reported in other studies. Further studies on virus accumulation in storage roots sprouts will provide more information on type of planting materials our farmers can use without risk of spreading viruses and loss of yield. Likewise, for virus elimination methods, the preferred explants to use are the shoot tips and a follow up of the findings after virus elimination will be documented once results are obtained.

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